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Exploitation of host cell biology and evasion of immunity by *Francisella tularensis*

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Francisella tularensis is an intracellular bacterium that infects humans and many small mammals. During infection, *F. tularensis* replicates predominantly in macrophages but also proliferate in other cell types. Entry into host cells is mediate by various receptors. Complement-opsonized *F. tularensis* enters into macrophages by looping phagocytosis. Uptake is mediated in part by Syk, which may activate actin rearrangement in the phagocytic cup resulting in the engulfment of *F. tularensis* in a lipid raft rich phagosome. Inside the host cells, *F. tularensis* resides transiently in an acidified late endosome-like compartment before disruption of the phagosomal membrane and escape into the cytosol, where bacterial proliferation occurs. Modulation of phagosome biogenesis and escape into the cytosol is mediated by the *Francisella* pathogenicity island-encoded type VI-like secretion system. Whilst inside the phagosome, *F. tularensis* temporarily induce proinflammatory cytokines in PI3K/Akt-dependent manner, which is counteracted by the induction of SHIP that negatively regulates PI3K/Akt activation and promotes bacterial escape into the cytosol. Interestingly, *F. tularensis* subverts CD4 T cells-mediated killing by inhibiting antigen presentation by activated macrophages through ubiquitin-dependent degradation of MHC II molecules on activated macrophages. In the cytosol, *F. tularensis* is recognized by the host cell inflammasome, which is down-regulated by *F. tularensis* that also inhibits caspase-1 and ASC activity. During late stages of intracellular proliferation, caspase-3 is activated but apoptosis is delayed through activation of NF-κB and Ras, which ensures cell viability.

Keywords: tularemia, ASC, caspase, apoptosis, Ras, Akt, SHIP

INFECTION BY *FRANCISELLA TULARENSIS*

Tularemia is a zoonotic disease caused by *Francisella tularensis*, a facultative intracellular pathogen that infects a broad range of small mammals and humans (Ellis et al., 2002; Pechous et al., 2009; Santic et al., 2010a). Four subspecies of *F. tularensis* have been identified to date (Keim et al., 2007; Nigrovic and Wingerter, 2008; Santic et al., 2009) and they share about 97% genomic identity (Champion et al., 2009; Larsson et al., 2009). These are subspecies *tularensis*, *holarctica*, *mediasiatica*, and *novicida*. Disease in humans is mostly caused by subspecies *tularensis* and *holarctica*. Subspecies *tularensis* is found in North America and is the most virulent, causing the most severe form of tularemia. In contrast subspecies *holarctica* is distributed throughout the northern hemisphere and causes a mild form of tularemia (Santic et al., 2006). Subspecies *novicida* does not cause disease in humans but causes a disease in mice that is similar to the disease in humans.

Francisella tularensis is transmitted to humans through inhalation of contaminated aerosol or ingestion of contaminated food and water, a bite by an arthropod vector, or direct contact with infected animals through skin abrasions (Ellis et al., 2002). Clinical presentation of disease depends on the route of infection and include pneumonic tularemia, oropharyngeal tularemia, and glandular or ulceroglandular tularemia (Ellis et al., 2002). Occasionally, *F. tularensis* can also infect the eye resulting in oculoglandular tularemia (Harrell and Whitaker, 1985). Ulceroglandular tularemia is characterized by an ulcer at the infected site with swelling of the regional lymph node. Glandular tularemia is similar to ulceroglandular but without the ulcer. In oropharyngeal tularemia the ulcer occurs in the mouth with swelling of the lymph nodes around the neck region. Irrespective of the route of infection the bacteria ultimately enter the blood stream, causing typhoidal tularemia, which leads to septicemia (Oyston et al., 2004; Nigrovic and Wingerter, 2008). Symptoms of the typhoidal tularemia include headache, fever, chills, nausea, diarrhea, and myalgia (Oyston et al., 2004; Nigrovic and Wingerter, 2008). Due to the high morbidity and mortality rate, the ease of dissemination and the fact that inhalation of as few as 10 organisms of subspecies *tularensis* can cause disease, *F. tularensis* has been classified by the CDC as a category A select agent.

Once inside the mammalian host, *F. tularensis* enters and replicates in macrophages (Anthony et al., 1991; Conlan and North, 1992; Fortier et al., 1994). However, there is increasing evidence that the organism can infect other cell types including neutrophils, dendritic cells, hepatocytes, and lung epithelial cells (Pechous et al., 2009). During infection, bacteria migrate from the initial site of infection to the liver and spleen where they replicate (Eigelsbach et al., 1962; Conlan et al., 2003). Although it has been shown that *F. tularensis* exhibit extracellular phase during *in vivo* infection in mice (Forestal et al., 2007; Yu et al., 2008), there is no data demonstrating extracellular growth during human or animal infection.

Available data indicate that intracellular trafficking of *F. tularensis* is similar in macrophages, neutrophils, epithelial cells, and *Drosophila melanogaster* S2 cells suggesting trafficking might be similar in all cell types (Golovliov et al., 2003a; Clemens et al., 2004; Santic et al., 2005a; McCaffrey and Allen, 2006; Craven et al., 2008; Santic et al., 2009).

F. tularensis enters into host cells through binding to surface receptors. This results in the uptake of the bacterium in a spacious loop by a mechanism referred to as looping phagocytosis (Clemens et al., 2005). Uptake by neutrophils and dendritic cells is dependent on opsonization (Lofgren et al., 1983; Ben Nasr et al., 2006) whereas entry into macrophages is through both opsonin dependent and independent mechanisms (Clemens et al., 2005; Balagopal et al., 2006; Pierini, 2006; Schulert and Allen, 2006; Barel et al., 2008). Inside the host cell, the bacteria reside transiently in a phagosome before escaping into the cytosol (**Figure 1**; Golovliov et al., 2003b; Clemens et al., 2004; Santic et al., 2005a,b; Checroun et al., 2006; Santic et al., 2007; Bonquist et al., 2008; Santic et al., 2008; Qin et al., 2009). Escape is preceded by modification of the phagosome to an acidified late endosome-like compartment (Fortier et al., 1995; Chong et al., 2008; Santic et al., 2008). Within this acidified compartment *F. tularensis* activates virulence genes that allow it to disrupt the phagosome membrane and escape into the cytosol (Chong et al., 2008; Santic et al., 2008).

Once inside the cytosol, the bacteria is recognized by the host cell inflammasome resulting in the cleavage of IL-1 and IL-18 (**Figure 1**; Mariathasan et al., 2005; Gavrilin et al., 2006; Henry et al., 2007; Fernandes-Alnemri et al., 2010; Jones et al., 2010). Similarly, there is activation of caspase-3 through both the extrinsic and intrinsic pathways between 6 and 12 post-infection. Although caspases are activated early during infection (Lai and Sjostedt, 2003; Mariathasan et al., 2005; Santic et al., 2010b), *F. tularensis* is able to delay death of the cells for its survival and replication by activating NF-κB and Ras both of which stimulate cells survival (Al-Khodor and Abu Kwaik, 2010; Santic et al., 2010b). During late stages of infection of mouse macrophages, *F. tularensis* is taken up in an autophagy-like compartment (Checroun et al., 2006). However, this re-entry of the *F. tularensis* into the endosomal–lysosomal pathway through autophagy does not occur in human macrophages, and therefore is not relevant to infection of humans (Akimana et al., 2010). Toward the end of the infectious cycle, the induction of apoptosis allows the bacteria to disrupt the cytoplasmic membrane and escape the spent cell to begin new infectious cycle (**Figure 1**).

ENTRY INTO AND REPLICATION WITHIN HOST CELLS

Francisella tularensis enters primary macrophages through both opsonin dependent and independent mechanisms. Complement-opsonized bacteria enter macrophages either through complement receptor 3 (CR3) or the scavenger receptor A (SRA)(Clemens et al., 2005; Pierini, 2006). Antibody-opsonized *F. tularensis* enters macrophages through FC gamma receptor (Balagopal et al., 2006) in contrast to unopsonized bacteria that enter macrophages through binding to the mannose receptor and surface nucleolin (Balagopal et al., 2006; Schulert and Allen, 2006; Barel et al., 2008). It has also been shown that opsonization of *F. tularensis* with lung collectin surfactant protein A (SP-A) enhance bacterial uptake by primary macrophages but the host cell receptor is not known (Balagopal et al., 2006). Similarly, the bacterial ligand for mannose receptor

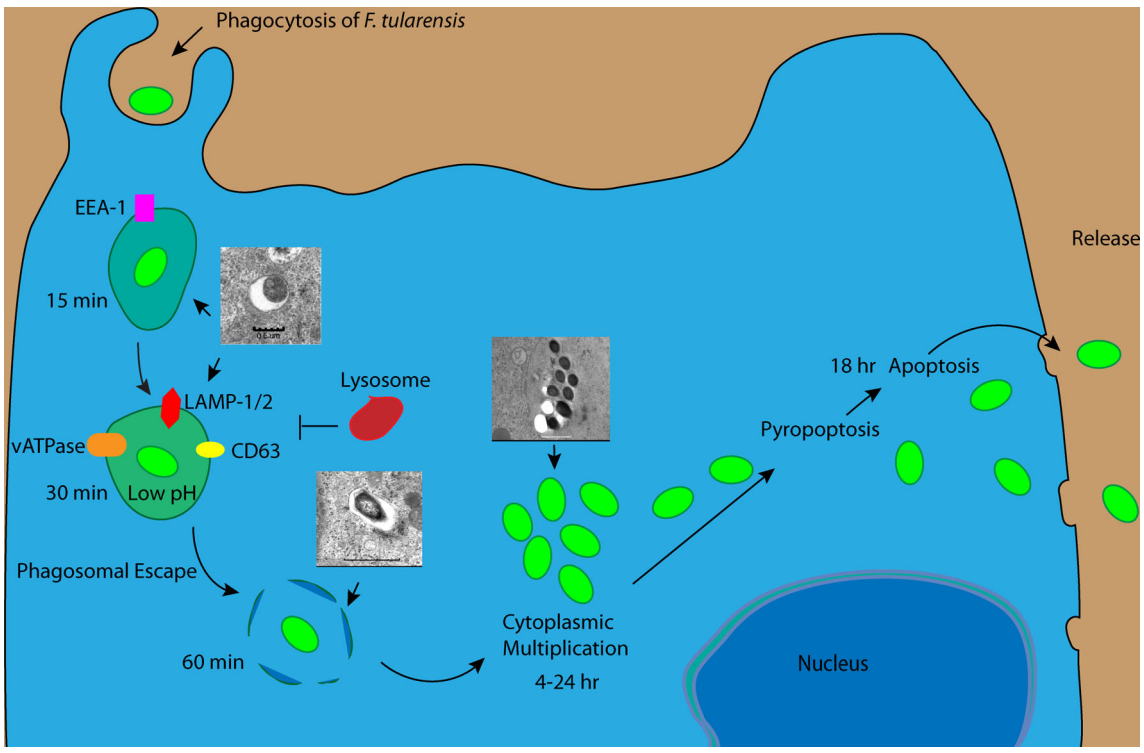

FIGURE 1 | Intracellular trafficking of *Francisella tularensis* within macrophages. *F. tularensis* enters macrophages using different receptors and resides transiently in the FCP, which acquires EE1 followed Lamp-1, Lamp-2, and Rab7 but excludes Cathepsin D. Within 30 min of infection the FCP acquires vATPase enabling *F. tularensis* to acidify the FCP and escape into the cytosol. Within the cytosol, *F. tularensis* activates caspase-1 and caspase-3 but delays pyropoptosis and apoptosis and maintain cell viability till late stages of infection when the bacteria exit the spent cell.

has not been identified. Elongation factor E2 is expressed on the surface of *F. tularensis* and bind to surface nucleolin expressed on the surface of macrophages (Barel et al., 2008). It is however not known which of these receptors are used predominantly *in vivo*.

When opsonized *F. tularensis* binds macrophages, it is engulfed in a unique asymmetric spacious pseudopod loops (Clemens et al., 2005). This unique mechanism of uptake has been shown to be dependent on intact CR3 and complement factor 3 (Clemens et al., 2005). Syk is important for Fcγ-mediated phagocytosis in macrophages and neutrophils (Greenberg et al., 1994; Raeder et al., 1999). Activation of Syk results in the activation of MAP kinase (ERKs) through Protein kinase C (PKC) leading to actin polymerization and induction of phagocytosis (Cox et al., 1996; Raeder et al., 1999). Syk has been shown to be important for the uptake of *F. tularensis* but the upstream receptor required for activation of Syk has not been identified (Parsa et al., 2008). Activation of Syk leads to subsequent activation of the Erk pathway but the direct binding partner of Syk is yet to be identified (**Figure 2**; Parsa et al., 2008). Interestingly, actin microfilament has been shown to be important for this process (Clemens et al., 2005).

In contrast to other intracellular bacteria such as *Salmonella typhimurium*, which requires PI3K to form the phagocytic cup, the uptake of *F. tularensis* is not affected by inhibition of the PI3K pathway (Parsa et al., 2006, 2008). This is consistent with a different mechanism used by *F. tularensis* to enter into host cells (Clemens et al., 2005). In addition to actin microfilament, the entry of *F. tularensis* into macrophages has been shown to be dependent on cholesterol-rich lipid domains known as lipid rafts since lipid rafts-associated components such as cholesterol and caveolin-1 are incorporated into the *Francisella*-containing phagosome (FCP) membrane upon its biogenesis from the macrophage plasma membrane (Tamilselvam and Daefler, 2008). The recruitment of lipid rafts to the FCP may act as a platform for linking the entry process of *F. tularensis* at the cell membrane to the cytoskeleton and the intracellular signaling pathways (Tamilselvam and Daefler, 2008).

To date, at least 268 gene products have been identified, that are important for replication of *F. tularensis* within mammalian cells (**Table 1**; Anthony et al., 1994; Baron and Nano, 1998;

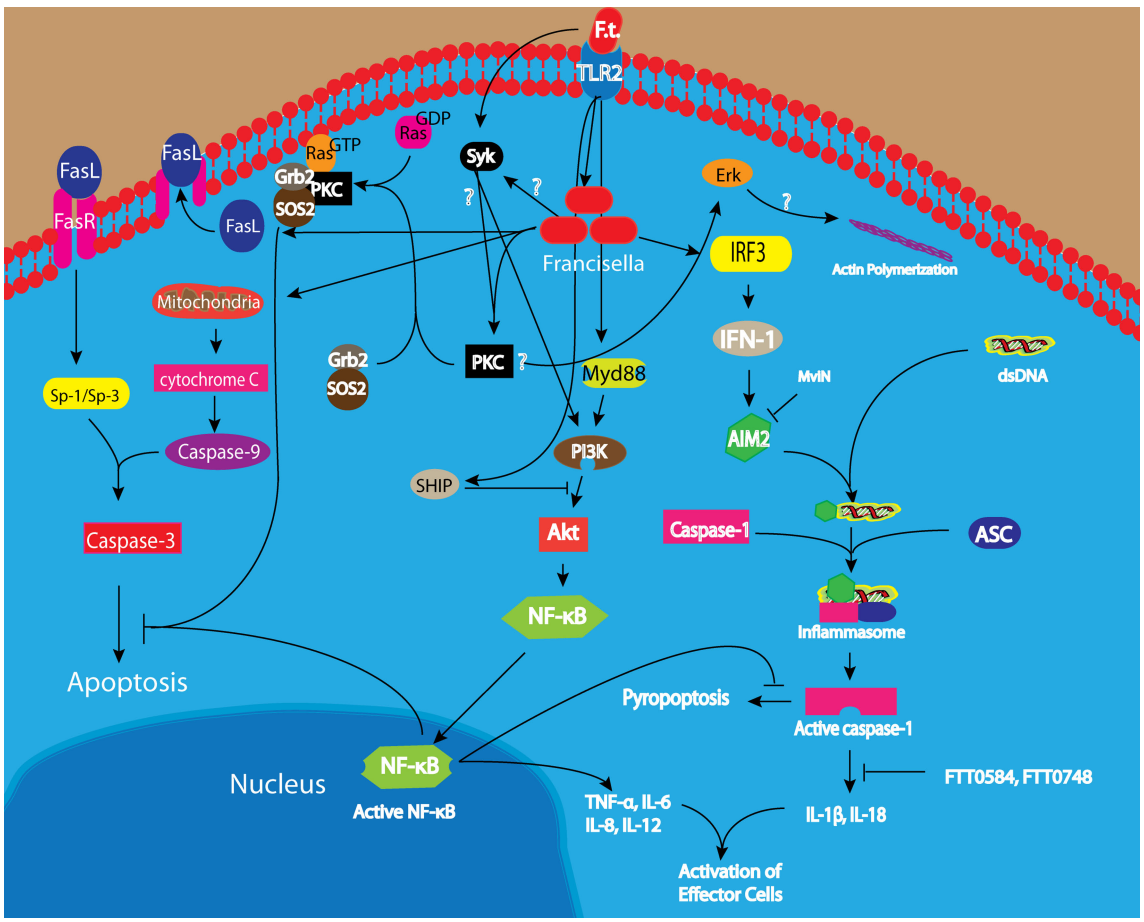

FIGURE 2 | Entry into and evasion of host cell innate immune response by *Francisella tularensis*. Phagocytosis of *F. tularensis* by macrophages is mediated by Syk-dependent activation of Erk, which likely triggers actin polymerization at the phagocytic cup. In addition, there is TLR2 dependent activation of Akt leading to induction of proinflammatory cytokines and phagosomal maturation. Akt activation is counteracted by SHIP activation, but the balance between the two opposing process is tilted toward escape of *F. tularensis* into the cytosol. Within the cytosol, *F. tularensis* activates both caspase-1 and caspase-3 but it is able to delay induction of apoptosis and pyropoptosis through Ras and NF-κB dependent anti-apoptotic mechanisms as well as AIM2-dependent inhibition of caspase-1 activation.

Table 1 | List of intracellular growth defective mutants.

PROTEINS OF UNKNOWN FUNCTION		
FTN_0027		Conserved protein of unknown function
FTN_0041		Protein of unknown function
FTN_0109		Protein of unknown function
FTN_0132		Protein of unknown function
FTN_0149		Conserved protein of unknown function
FTN_0275		Conserved protein of unknown function
FTN_0290		Protein of unknown function
FTN_0297		Conserved protein of unknown function
FTN_0428		Protein of unknown function
FTN_0444		Membrane protein of unknown function
FTN_0477		Conserved protein of unknown function
FTN_0788		Conserved protein of unknown function
FTN_0855		Protein of unknown function
FTN_0915		Conserved protein of unknown function
FTN_0925		Protein of unknown function
FTN_0930		Protein of unknown function
FTN_0933		Protein of unknown function
FTN_0977		Conserved protein of unknown function
FTN_1170		Conserved protein of unknown function
FTN_1172		Conserved protein of unknown function
FTN_1175		Membrane protein of unknown function
FTN_1256		Membrane protein of unknown function
FTN_1343		Conserved protein of unknown function
FTN_1367		Protein of unknown function
FTN_1457		Protein of unknown function
FTN_1542		Conserved protein of unknown function
FTN_1624		Conserved protein of unknown function
FTN_1696		Protein of unknown function
FTN_1713		Protein of unknown function
FTN_1764		Protein of unknown function
HYPOTHETICAL PROTEINS		
FTL_0439		Hypothetical outer membrane protein
FTL_0544		Hypothetical protein; polyphosphate kinase
FTL_0706		Hypothetical membrane protein; LPS biosynthesis
FTL_0886		Conserved hypothetical protein YleA; possible tRNA-i (6)A37 methylthiotransferase
FTL_1096		Hypothetical lipoprotein; ABC transporter and potential disulfide bond formation
FTL_1414		Hypothetical protein; possible capsule-related protein
FTN_0030		Hypothetical membrane protein
FTN_0038		Hypothetical protein
FTN_0169		Conserved hypothetical membrane protein
FTN_0336		Hypothetical protein
FTN_0384		Conserved hypothetical protein
FTN_0403		Hypothetical membrane protein
FTN_0534		Conserved hypothetical membrane protein
FTN_0556		Hypothetical protein
FTN_0696		Hypothetical membrane protein
FTN_0709		Hypothetical protein
FTN_0727		Hypothetical membrane protein
FTN_0759		Conserved hypothetical protein
FTN_0792		Hypothetical protein
FTN_0847		Conserved hypothetical protein
FTN_0888		Hypothetical membrane protein
FTN_0895		Hypothetical protein
FTN_1098		Conserved hypothetical membrane protein
FTN_1156		Hypothetical protein
FTN_1349		Hypothetical protein
FTN_1395		Conserved hypothetical protein
FTN_1406		Conserved hypothetical membrane protein
FTN_1612		Hypothetical protein
FTN_1656		Conserved hypothetical protein
FTN_1686		Hypothetical membrane protein
FTN_1736		Hypothetical protein
FTT1103		Conserved hypothetical lipoprotein
FTT1236		Hypothetical protein
FTT1244c	yfiO	Conserved hypothetical lipoprotein
FPI PROTEINS		
FTN_1309	pdpA	Protein of unknown function
FTN_1310	icmF	Conserved protein of unknown function
FTN_1311	iglE	Protein of unknown function
FTN_1312	vgrG	Conserved hypothetical protein
FTN_1313	iglF	Hypothetical protein
FTN_1314	iglG	Conserved hypothetical protein
FTN_1315	iglH	Protein of unknown function
FTN_1316	dotU	Conserved protein of unknown function
FTN_1317	iglI	Protein of unknown function
FTN_1318	iglJ	Hypothetical protein
FTN_1321	iglD	Intracellular growth locus, subunit D
FTN_1322	iglC	Intracellular growth locus, subunit C
FTN_1323	iglB	Intracellular growth locus protein B
FTN_1324	iglA	Intracellular growth locus A
FTN_1325	pdpD	Protein of unknown function
METABOLIC PROTEINS		
FTL_0028	pryB	Aspartate carbamoyltransferase
FTL_0029	carB	Carbamoyl-phosphate synthase large chain
FTL_0030	carA	Carbamoyl-phosphate synthase small chain
FTL_0483	*glgB*	Glycogen branching enzyme, GlgB; polysaccharide metabolism
FTL_0592	*wbtA*	dTDP-glucose 4,6-dehydratase, WbtA, O-antigen polysaccharide biosynthesis
FTL_0594	*wbtC*	UDP-glucose-4-epimerase, WbtC, O-antigen polysaccharide biosynthesis
FTL_0606	*wbtM*	dTDP-glucose 4,6-dehydratase, WbtM, O-antigen polysaccharide biosynthesis
FTL_0766	*ggt*	Gamma-glutamyl transpeptidase; amino acid, arachidonic acid, and glutathione
FTL_0789	*aspC2*	Aspartate aminotransferase; amino acid biosynthesis
FTL_1262		Chorismate family binding protein; aromatic amino acid, and folate biosynthesis
FTL_1415	*capC*	Capsule biosynthesis protein CapC
FTL_1416	*capB*	Capsule biosynthesis protein CapB
FTN_0020	carB	Carbamoyl-phosphate synthase large chain
FTN_0035	pyrF	Orotidine-5-phosphate decarboxylase

(Continued)

Table 1 | Continued

FTN_0036	pyrD	Dihydroorotate oxidase
FTN_0063	ilvE	Branched-chain amino acid aminotransferase protein (class IV)
FTN_0090	acpA	Acid phosphatase
FTN_0111	ribH	Riboflavin synthase beta-chain
FTN_0113	ribC	Riboflavin synthase alpha chain
FTN_0125	ackA	Propionate kinase 2/acetate kinase A
FTN_0178	purA	Adenylosuccinate synthetase
FTN_0199	cyoE	Heme O synthase
FTN_0211	pcp	Pyrrolidone carboxylate peptidase
FTN_0218	nfnB	Dihydropteridine reductase
FTN_0319		Amino acid–polyamine–organocation family protein
FTN_0343		Aminotransferase
FTN_0358		tRNA-methylthiotransferase MiaB protein
FTN_0420		SAICAR synthetase/ phosphoribosylamine-glycine ligase
FTN_0483		Bifunctional NMN adenylyltransferase/nudix hydrolase
FTN_0496	Slt	Soluble lytic murein transglycosylase
FTN_0504		Lysine decarboxylase
FTN_0507	gcvP1	Glycine cleavage system P protein, subunit 1
FTN_0511		Shikimate 5-dehydrogenase
FTN_0524	Asd	Aspartate semialdehyde dehydrogenase
FTN_0527	thrC	Threonine synthase
FTN_0545		Glycosyl transferase, group 2
FTN_0567		tRNA synthetase class II (D, K, and N)
FTN_0588		Asparaginase
FTN_0593	sucD	Succinyl-CoA synthetase, alpha subunit
FTN_0598		tRNA-dihydrouridine synthase
FTN_0633	katG	Peroxidase/catalase
FTN_0692	nadA	Quinolinate synthetase A
FTN_0695	Add	Deoxyadenosine deaminase/adenosine deaminase
FTN_0746	Alr	Alanine racemase
FTN_0806		Glycosyl hydrolase family 3
FTN_0811	birA	Biotin—acetyl-CoA-carboxylase ligase
FTN_0822		*p*-Aminobenzoate synthase component I
FTN_0840	mdaB	NADPH-quinone reductase (modulator of drug activity B)
FTN_0877	cls	Cardiolipin synthetase
FTN_0928	cysD	Sulfate adenylyltransferase subunit 2
FTN_0954		Histidine acid phosphatase
FTN_0957		Short chain dehydrogenase
FTN_0965		Metal-dependent exopeptidase
FTN_0983		Bifunctional protein: glutaredoxin 3/ribonucleotide reductase beta subunit
FTN_0988	prmA	50S ribosomal protein L11, methyltransferase
FTN_0995	hslV	ATP-dependent protease HslVU, peptidase subunit
FTN_1018		Aldolase/adducin class II family protein
FTN_1046	wzb	Low molecular weight (LMW) phosphotyrosine protein phosphatase
FTN_1061		Acid phosphatase, HAD superfamily protein
FTN_1121	phrB	Deoxyribodipyrimidine photolyase
FTN_1131	putA	Bifunctional proline dehydrogenase, pyrroline-5-carboxylate dehydrogenase
FTN_1135	aroB	3-Dehydroquinate synthetase
FTN_1222	kpsF	Phosphosugar isomerase
FTN_1231	gloA	Lactoylglutathione lyase
FTN_1233		Haloacid dehalogenase-like hydrolase
FTN_1234	queA	S-adenosylmethionine: tRNA ribosyltransferase-isomerase
FTN_1333	tktA	Transketolase I
FTN_1415		Thioredoxin
FTN_1417	manB	Phosphomannomutase
FTN_1421	wbtH	Glutamine amidotransferase/asparagine synthase
FTN_1428	wbtO	Transferase
FTN_1494	aceE	Pyruvate dehydrogenase complex, E1 component, pyruvate dehydrogenase
FTN_1523		Amino acid–polyamine–organocation family protein
FTN_1553	nudH	dGTP pyrophosphohydrolase
FTN_1557		Oxidoreductase iron/ascorbate family protein
FTN_1584	glpD	Glycerol-3-phosphate dehydrogenase
FTN_1585	glpK	Glycerol kinase
FTN_1597	prfC	Peptide chain release factor 3
FTN_1619	appC	Cytochrome bd-II terminal oxidase subunit I
FTN_1620	appB	Cytochrome bd-II terminal oxidase subunit II
FTN_1621		Predicted NAD/FAD-dependent oxidoreductase
FTN_1655	rluC	Ribosomal large subunit pseudouridine synthase C
FTN_1701		Glutamate decarboxylase
FTN_1767	rbsK	Ribokinase, pfkB family
FTN_1777	trpG	Anthranilate synthase component II
FTT0203c	purH	Bifunctional purine biosynthesis protein
FTT0204	purA	Adenylosuccinate synthetase
FTT0435	Ctu	Citrulline ureidase
FTT0588	aroA	3-Phosphoshikimate 1-carboxyvinyl transferase
FTT1234		Choloylglycine hydrolase family protein
FTT1665	purL	Aspartate carbamoyltransferase
FTT1720c		Phosphoribosylformylglycinamidine synthase
FTT1721c	purF	(Amidophosphoribosyltransferase)2
FTT1762c		Acetyltransferase protein
FTL_1071	guaA	GMP synthase (glutamine-hydrolyzing)
FTL_1478	guaB	Inosine-5-monophosphate dehydrogenase
TRANSPORTER PROTEINS		
FTL_0304		Na+/H+ antiporter; regulation of pH
FTL_0837	*metIQ*	D-Methionine transport protein (ABC transporter), MetIQ
FTL_0838	*metN*	D-Methionine transport protein (ABC transporter), MetN
FTL_1583	*xasA*	Glutamate–aminobutyric acid antiporter, XasA; amino acid transport
FTL_1806		Major facilitator superfamily transporter
FTN_0008		10 TMS drug/metabolite exporter protein
FTN_0018	sdaC	Serine permease

(Continued)

Table 1 | Continued

FTN_0141		ABC transporter, ATP-binding protein
FTN_0299	putP	Proline: Na+ symporter
FTN_0619		Pseudogene: nicotinamide ribonucleoside (NR) uptake permease (PnuC) family protein
FTN_0624		Serine permease
FTN_0636	glpT	Glycerol-3-phosphate transporter
FTN_0687	galP1	Galactose-proton symporter, major facilitator superfamily (MFS) transport protein
FTN_0728		Predicted Co/Zn/Cd cation transporter
FTN_0739	potG	ATP-binding cassette putrescine uptake system, ATP-binding protein
FTN_0799	emrE	Putative membrane transporter of cations and cationic drugs, multidrug resistance protein
FTN_0800		ArsB arsenite/antimonite exporter
FTN_0848		Amino acid antiporter
FTN_0885		Proton-dependent oligopeptide transporter (POT) family protein, di-, or tripeptide: H+ symporter
FTN_0997		Proton-dependent oligopeptide transporter (POT) family protein, di-, or tripeptide: H+ symporter
FTN_1215	kpsC	Capsule polysaccharide export protein KpsC
FTN_1344		Major facilitator superfamily (MFS) transport protein
FTN_1368	feoA	Fe_2 transport system protein A
FTN_1441		Sugar porter (SP) family protein
FTN_1581		Small conductance mechanosensitive ion channel (MscS) family protein
FTN_1593	oppA	ABC-type oligopeptide transport system, periplasmic component
FTN_1611		Major facilitator superfamily (MFS) transport protein
FTN_1711	tyrP	Tyrosine permease
FTN_1716	kdpC	Potassium-transporting ATPase C chain
FTN_1716	kdpC	Potassium-transporting ATPase C chain
FTN_1733		Nicotinamide ribonucleoside (NR) uptake permease (PnuC) family protein
FTT0056c		Major facilitator super family (MFS) transport protein
FTT0129		Major facilitator super family (MFS) transport protein
DNA MODIFYING		
FTL_0878		DNA/RNA endonuclease family
FTN_0133		Ribonuclease II family protein
FTN_0287		Type I restriction-modification system, subunit R (restriction)
FTN_0577	mutL	DNA mismatch repair enzyme with ATPase activity
FTN_0680	uvrC	Excinuclease ABC, subunit C
FTN_0710		Type I restriction-modification system, subunit R (restriction)
FTN_0838	xthA	Exodeoxyribonuclease III
FTN_1017		Pseudogene: DNA-3-methyladenine glycosylase
FTN_1027	ruvC	Holliday junction endodeoxyribonuclease
FTN_1073		DNA/RNA endonuclease G
FTN_1154		Type I restriction–modification system, subunit S
FTN_1176	uvrB	Excinuclease ABC, subunit B
FTN_1197	recR	RecFOR complex, RecR component
FTN_1293	rnhB	Ribonuclease HII
FTN_1357	recB	ATP-dependent exoDNase_subunit
FTN_1487		Restriction endonuclease
TRANSCRIPTION/TRANSLATION		
FTL_1542	migR	Macrophage intracellular growth regulator
FTL_1606	sspA	Stringent starvation protein A/regulator of transcription
FTL_1914	ripA	Required for intracellular proliferation, factor A
FTN_0480	fevR	*Francisella* effector of virulence regulation
FTN_0567		tRNA synthetase class II (D, K, and N)
FTN_0598		tRNA-dihydrouridine synthase
FTN_1290	mglA	Macrophage growth locus, protein A
FTN_1291	mglB	Macrophage growth locus, subunit B
FTN_1412		DNA-directed RNA polymerase subunit
FTL_0552		Transcriptional response regulator
CELL DIVISION		
FTN_0162	ftsQ	Cell division protein FtsQ
FTN_0330	minD	Septum formation inhibitor-activating ATPase
FTN_0331	minC	Septum formation inhibitor-activating ATPase
TYPE IV PILIN		
FTN_0415	pilA	Type IV pili, pilus assembly protein
FTN_1137	pilQ	Type IV pili secretin component
FTN_1139	pilO	Type IV pili glycosylation protein
OTHERS		
FTL_0094	clpB	ClpB protein
FTL_1670	*dsbB*	Disulfide bond formation protein, DsbB
FTN_0107	lepA	GTP-binding protein LepA
FTN_0155		Competence protein
FTN_0182		ATP-binding cassette (ABC) superfamily protein
FTN_0286		Transposase
FTN_0338		MutT/nudix family protein
FTN_0465		Sua5/YciO/YrdC family protein
FTN_0646	cscK	ROK family protein
FTN_0672	secA	Preprotein translocase, subunit A (ATPase, RNA helicase)
FTN_0768	tspO	Tryptophan-rich sensory protein
FTN_0985		DJ-1/PfpI family protein
FTN_1002	blaA	Beta-lactamase class A
FTN_1031	ftnA	Ferric iron binding protein, ferritin-like
FTN_1034	rnfB	Iron–sulfur cluster-binding protein
FTN_1058	tig	Trigger factor (TF) protein
FTN_1064		PhoH family protein, putative ATPase
FTN_1145	era	GTP-binding protein
FTN_1217		ATP-binding cassette (ABC) superfamily protein
FTN_1240		BolA family protein
FTN_1241		DedA family protein
FTN_1263	comL	Competence lipoprotein ComL
FTN_1355		Regulatory factor, Bvg accessory factor family
FTN_1518	relA	GDP pyrophosphokinase/GTP pyrophosphokinase
FTT0029c	figA	*Francisella* iron regulated gene A
FTT0918		
FTL_0380	sodC	Superoxide dismutase (Cu–Zn) precusor
FTL_0439	fupA/B	Siderophore biosynthesis

Gray et al., 2002; Golovliov et al., 2003a; Nano et al., 2004; Santic et al., 2005b, 2007; Twine et al., 2005; Deng et al., 2006; Pechous et al., 2006, 2008; Tempel et al., 2006; Charity et al., 2007; de Bruin et al., 2007; Maier et al., 2007; Mohapatra et al., 2007a,b, 2008; Raynaud et al., 2007; Bonquist et al., 2008; Brotcke and Monack, 2008; Fuller et al., 2008; Meibom et al., 2008; Sammons-Jackson et al., 2008; Alkhuder et al., 2009; Buchan et al., 2009; Dean et al., 2009; Mahawar et al., 2009; Santiago et al., 2009; Schulert et al., 2009; Ahlund et al., 2010; Asare and Abu Kwaik, 2010; Jia et al., 2010; Sen et al., 2010). These include the *Francisella* pathogenicity Island (FPI) proteins IglA, IglB, IglC, IglD, pdpA, pdpB, pdpD and their regulators, MglA, SspA, FevR, MigR, RipA, PigR, and PmrA (Baron and Nano, 1998; Gray et al., 2002; Golovliov et al., 2003a; Charity et al., 2007, 2009; de Bruin et al., 2007; Mohapatra et al., 2007b; Bonquist et al., 2008; Brotcke and Monack, 2008; Fuller et al., 2008; Buchan et al., 2009). The FPI is composed of 17 genes and recent mutagenesis experiments have shown that most of the genes are important for survival within the host cell (Golovliov et al., 2003a; de Bruin et al., 2007; Barker et al., 2009; Broms et al., 2009; Schmerk et al., 2009). Some of the gene products on the FPI form a type VI-like secretion system through which effector proteins are injected into the host cell cytosol to modulate biogenesis of the FCP and to enable the bacterium to disrupt the phagosome membrane and escape into the cytosol (Golovliov et al., 2003a; Santic et al., 2007; Barker et al., 2009; Broms et al., 2009; Schmerk et al., 2009). MglA, SspA, and PmrA bind cooperatively with RNA polymerase to regulate a large number of genes including those of the FPI (Brotcke et al., 2006; Charity et al., 2007; Mohapatra et al., 2007b; Bell et al., 2010) that are important for survival within the host cell. This regulation is mediated in part by FevR which is important for escape and replication within the cytosol (Brotcke and Monack, 2008). FevR is also independently regulated by MigR (Buchan et al., 2009) indicating that FevR plays a central role in the regulation of virulence in *F. tularensis*. Independent of FevR, MglA, and SspA also regulate virulence genes through cooperative interaction with PigR and the alamone ppGpp (Charity et al., 2009). Whereas most of the genes regulated by the different pathways are common, there are subsets of genes that are regulated independently by the different pathways (Brotcke et al., 2006; Charity et al., 2007; Mohapatra et al., 2007b). A large number of these gene products and most of the proteins that are necessary for escape and replication are hypothetical proteins. It is conceivable to speculate that some of these gene products constitute effector proteins that are secreted by the type VI secretion-like system. Also important for intracellular replication are genes involved in the transport of metabolic intermediates and different metabolic pathways including amino acid metabolism, nucleotide metabolism, and carbohydrate metabolism (Pechous et al., 2006; Alkhuder et al., 2009; Mahawar et al., 2009; Schulert et al., 2009; Asare and Abu Kwaik, 2010). The large number of metabolic genes that is required for replication indicates that the FCP is replete of nutrients and *F. tularensis* require *de novo* synthesis in order to survive and replicate within the host cells. Once in the cytosol where nutrient is readily available *F. tularensis* may acquire nutrients through the importation of metabolic intermediates from the host cell cytosol. This may explain why mutations in a large number of metabolic intermediate transporters block bacterial escape into the cytosol (**Table 1**; Qin and Mann, 2006; Maier et al., 2007; Asare and Abu Kwaik, 2010).

MODULATION OF PHAGOSOME BIOGENESIS

Phagosomal maturation involves sequential interaction between the nascent phagosome and the endocytic and lysosomal vesicles resulting in the conversion of the phagosome to a phagolysosome within which the bacterium or a particle is degraded (Duclos and Desjardins, 2000; Hackstadt, 2000; Kahn et al., 2002). After biogenesis from the plasma membrane, the nascent phagosome fuses with vesicles from the early endosome in a process that is regulated by Rab5 GTPase and the downstream effector early endosomal antigen 1 (EEA1). This is followed by interaction with the late endosome that is controlled by Rab7 GTPase. The late endosome-like phagosome becomes acidified through acquisition of the vacuolar ATPase, which pumps protons into the lumen of the phagosome resulting in acidification of the lumen. The acidified phagosome subsequently fuses to the lysosomes to form a phagolysosome, which is very rich in acid hydrolases. Within this compartment the microbe or particle is degraded (Duclos and Desjardins, 2000; Hackstadt, 2000; Kahn et al., 2002; **Figure 1**). The maturation process is very rapid and is completed within 15–30 min of phagosome biogenesis from the plasma membrane (Duclos and Desjardins, 2000; Hackstadt, 2000; Kahn et al., 2002).

Different intracellular pathogens have evolved different mechanisms to subvert the default endocytic maturation to create permissive niches that allow intracellular replication (Duclos and Desjardins, 2000; Hackstadt, 2000; Kahn et al., 2002). The strategies include (i) Arrest of phagosome maturation at a distinct stage in the endosomal–lysosomal degradation pathway, as occurs in infection with *Legionella pneumophila*; (ii) Survival and replication within an acidic environment of a mature phagolysosomes, as exemplified by *Coxiella burnetii*; and (iii) Replication within the cytosol after degradation of the phagosomal membrane, as occurs in infection with *Listeria monocytogenes* and *Shigella flexneri* (Duclos and Desjardins, 2000; Hackstadt, 2000; Kahn et al., 2002). Although the endocytic maturation stage of phagosome harboring vacuolar pathogens has been classified into early or late endosome, maturation of phagosome harboring intracellular pathogens is aberrant and is not a classical full maturation of any of the defined endocytic stages (Santic et al., 2010a). For example, the *Mycobacterium tuberculosis* phagosome acquires Rab5 but lacks several downstream effectors of Rab5 that are present on mature early endosome. It also acquires procathepsin D, which is the immature form of the lysosomal enzyme cathepsin D (Sturgill-Koszycki et al., 1996; Derre and Isberg, 2004). Therefore, it might be more accurate to classify phagosome of intracellular vacuolar pathogens as early endosome-like or late endosome-like phagosome (Santic et al., 2010a).

The FCP transiently acquires the EEA1 followed by the acquisition of the late endosomal markers, Lamp1/2, Cd63, and Rab7 as well as the vacuolar ATPase, which acidifies the phagosome (**Figure 1**; Golovliov et al., 2003b; Clemens et al., 2004; Santic et al., 2005a,b, 2007, 2008; Checroun et al., 2006; Bonquist et al., 2008; Qin et al., 2009). The FCP does not however co-localize with the lysosomal acid hydrolase cathepsin D and the fluid face marker, lysotraker

(**Figure 1**; Chong et al., 2008; Santic et al., 2008). Within 30–60 min, the bacterium disrupts the phagosomal membrane and escapes into the host cell cytosol (**Figure 1**; Chong et al., 2008; Santic et al., 2008). Acidification of the vacuole is important for the ability of the bacteria to escape into the cytosol, since inhibition of the vATPase results in a delay in bacterial escape into the cytosol (Chong et al., 2008; Santic et al., 2008). *F. tularensis* has been shown to escape into the cytosol in different cell types including macrophages and neutrophils (**Figure 1**; Golovliov et al., 2003a; Clemens et al., 2004; Santic et al., 2005a; McCaffrey and Allen, 2006). It has not been determined if *F. tularensis* arrest phagosome maturation before escaping into cytosol or if the bacterium manages to escape before the phagosome fuses to the lysosome. However, trafficking of the *migR* and *fevR* mutants of *F. tularensis* in macrophages suggests that there is arrest of phagosome biogenesis prior to bacterial escape into the cytosol (Buchan et al., 2009). Comparison of trafficking of the *migR* and *fevR* mutants showed that whereas the *fevR* mutant is trapped in a LAMP1-positive compartment, the phagosome containing the *migR* mutant matures into a phagolysosome enriched in LAMP1 and cathepsin D (Buchan et al., 2009). Conversely, data from studies of *F. tularensis* trafficking in neutrophils suggest that a fraction of the phagosome of wild-type bacteria that are unable to escape end up in a phagolysosome. This may suggest that *F. tularensis* does not inhibit phagosome maturation in neutrophils but rather escape into the cytosol before the phagosome matures into a phagolysosome (McCaffrey and Allen, 2006). Interestingly, arrest in phagosome biogenesis and rapid escape of *F. tularensis* into the cytosol is also exhibited in arthropod vector-derived cells, indicating exploitation of conserved eukaryotic processes by *F. tularensis* to infect and proliferate within arthropod and mammalian cells (Santic et al., 2009).

ESCAPE INTO THE CYTOSOL

Like other intracellular pathogens, *F. tularensis* must overcome the host innate immune response to successfully colonize the intracellular niche. Since the primary host defense is centered on the antimicrobial properties of the phagosome, *F. tularensis* like other cytosolic bacteria escapes from the phagosome into the cytosol where it replicates (Goebel and Kuhn, 2000; Golovliov et al., 2003a; Clemens et al., 2004; Santic et al., 2005a; McCaffrey and Allen, 2006; Ray et al., 2009). In order to escape into the cytosol, the FCP transiently acquires the vacuolar ATPase, which acidifies the phagosome followed by rapid escape of *F. tularensis* to the cytosol (**Figure 1**; Golovliov et al., 2003b; Clemens et al., 2004; Santic et al., 2005a,b, 2007, 2008; Checroun et al., 2006; Bonquist et al., 2008; Qin et al., 2009). The acidification is important since inhibition of the vATPase by bafilomycin A delays escape of the bacterium into the host cell cytosol indicating that there is a factor involved in disruption of the phagosome that is expressed or activated at acidic pH. Between 15 and 30 min of residence in the phagosome in human macrophages, the bacteria begin to escape into the cytosol (**Figure 1**; Santic et al., 2010a). It is within the cytosol that the bacteria replicate (**Figure 1**). The mechanism by which the bacterium escapes into the cytosol is not well understood.

Unlike *L. monocytogenes*, a large number of genes have been shown to be important for escape of *F. tularensis* into the host cell cytosol (**Table 2**; Golovliov et al., 2003a; Santic et al., 2005b;

Table 2 | List of escape defective mutants.

PROTEINS OF UNKNOWN FUNCTION		
FTN_0027		Conserved protein of unknown function
FTN_0109		Protein of unknown function
FTN_0149		Conserved protein of unknown function
FTN_0297		Conserved protein of unknown function
FTN_0444		Membrane protein of unknown function
FTN_0788		Conserved protein of unknown function
FTN_0855		Protein of unknown function
FTN_0915		Conserved protein of unknown function
FTN_0925		Protein of unknown function
FTN_0930		Protein of unknown function
FTN_0933		Protein of unknown function
FTN_0977		Conserved protein of unknown function
FTN_1175		Membrane protein of unknown function
FTN_1256		Membrane protein of unknown function
FTN_1343		Conserved protein of unknown function
FTN_1624		Conserved protein of unknown function
FTN_1764		Protein of unknown function
HYPOTHETICAL PROTEINS		
FTN_0030		Hypothetical membrane protein
FTN_0038		Hypothetical protein
FTN_0096		Conserved hypothetical membrane protein
FTN_0403		Hypothetical membrane protein
FTN_0727		Hypothetical membrane protein
FTN_0792		Hypothetical protein
FTN_0847		Conserved hypothetical protein
FTN_1098		Conserved hypothetical membrane protein
FTN_1349		Hypothetical protein
FTN_1395		Conserved hypothetical protein
FTN_1406		Conserved hypothetical membrane protein
FTN_1612		Hypothetical protein
FTN_1686		Hypothetical membrane protein
FTT1103		Conserved hypothetical lipoprotein
FPI PROTEINS		
FTN_1309	pdpA	Protein of unknown function
FTN_1313	vgrG	
FTN_1317	iglI	Intracellular growth locus, subunit I
FTN_1322	iglC	Intracellular growth locus, subunit C
FTN_1323	iglB	Intracellular growth locus protein B
FTN_1324	iglA	Intracellular growth locus A
FTN_1325	pdpD	Protein of unknown function
METABOLIC PROTEINS		
FTN_0019	pyrB	Aspartate carbamoyltransferase
FTN_0063	ilvE	Branched-chain amino acid aminotransferase protein (class IV)
FTN_0090		Acid phosphatase
FTN_0125	ackA	Propionate kinase 2/acetate kinase A
FTN_0483		Bifunctional NMN adenylyltransferase/Nudix hydrolase
FTN_0504		Lysine decarboxylase
FTN_0511		Shikimate 5-dehydrogenase
FTN_0524	asd	Aspartate semialdehyde dehydrogenase

(Continued)

Table 2 | Continued

FTN_0527	thrC	Threonine synthase
FTN_0545		Glycosyl transferase, group 2
FTN_0692	nadA	Quinolinate synthetase A
FTN_0746	alr	Alanine racemase
FTN_0811	birA	Biotin–acetyl-CoA-carboxylase ligase
FTN_0822		*p*-Aminobenzoate synthase component I
FTN_0840	mdaB	NADPH-quinone reductase (modulator of drug activity B)
FTN_0877	cls	Cardiolipin synthetase
FTN_0954		Histidine acid phosphatase
FTN_0965		Metal-dependent exopeptidase
FTN_0983		Bifunctional protein: glutaredoxin 3/ribonucleotide reductase beta subunit
FTN_0988	prmA	50S ribosomal protein L11, methyltransferase
FTN_1061		Acid phosphatase, HAD superfamily protein
FTN_1222	kpsF	Phosphosugar isomerase
FTN_1231	gloA	Lactoylglutathione lyase
FTN_1234	queA	S-adenosylmethionine: tRNA ribosyltransferase-isomerase
FTN_1333	tktA	Transketolase I
FTN_1376		
FTN_1418	manC	
FTN_1428	wbtO	Transferase
FTN_1494	aceE	Pyruvate dehydrogenase complex, E1 component, pyruvate dehydrogenase
FTN_1553	nudH	dGTP pyrophosphohydrolase
FTN_1597	prfC	Peptide chain release factor 3
FTN_1621		Predicted NAD/FAD-dependent oxidoreductase
FTN_1655	rluC	Ribosomal large subunit pseudouridine synthase C
TRANSPORTER PROTEINS		
FTN_0624		Serine permease
FTN_0728		Predicted Co/Zn/Cd cation transporter
FTN_0997		Proton-dependent oligopeptide transporter (POT) family protein, di-, or tripeptide: H+ symporter
FTN_1344		Major facilitator superfamily (MFS) transport protein
FTN_1611		Major facilitator superfamily (MFS) transport protein
FTN_1711	tyrP	Tyrosine permease
DNA MODIFICATION		
FTN_0133		Ribonuclease II family protein
FTN_0680	uvrC	Excinuclease ABC, subunit C
FTN_0710		Type I restriction–modification system, subunit R (restriction)
FTN_1027	ruvC	Holliday junction endodeoxyribonuclease
FTN_1073		DNA/RNA endonuclease G
FTN_1154		Type I restriction–modification system, subunit S
TRANSCRIPTION/TRANSLATION		
FTN_1290	mglA	Macrophage growth locus, protein A
FTL_1542	migR	Macrophage intracellular growth regulator
FTL_1914	ripA	Required for intracellular proliferation, factor A
FTN_0480	fevR	*Francisella* effector of virulence regulation
TYPE IV PILIN		
FTN_1137	pilQ	Type IV pili secretin component
FTN_1139	pilO	Type IV pili glycosylation protein
OTHERS		
FTN_0286		Transposase
FTN_0646	cscK	ROK family protein
FTN_0768	tspO	Tryptophan-rich sensory protein
FTN_1034	rnfB	Iron–sulfur cluster-binding protein
FTN_1145	era	GTP-binding protein
FTN_1241		DedA family protein
FTN_1263	comL	Competence lipoprotein ComL
FTN_1453		Two-component regulator, sensor histidine kinase
FTN_1518	relA	GDP pyrophosphokinase/GTP pyrophosphokinase

Qin and Mann, 2006; Mohapatra et al., 2008; Barker et al., 2009; Broms et al., 2009; Buchan et al., 2009; Schmerk et al., 2009; Schulert et al., 2009; Asare and Abu Kwaik, 2010). Recent mutagenesis experiments have shown that most of the genes of the FPI that form the type VI-like secretion system, affect escape of the bacterium into the cytosol and subsequent replication (Golovliov et al., 2003a; de Bruin et al., 2007; Barker et al., 2009; Broms et al., 2009; Schmerk et al., 2009). In contrast, IglD has been shown to be important for replication of the bacteria within the cytosol without any effect on phagosomal escape of the bacterium (Santic et al., 2007). The FPI protein VgrG has been shown to be a component of the secretary system as well as a substrate of the system (Barker et al., 2009). Unlike VgrG, IglI is a substrate of the type VI secretion system with no effect on the secretion apparatus. Both genes are important for escape of *F. tularensis* into the host cell cytosol but the mechanism of action has not been elucidated (Barker et al., 2009). The FPI proteins IglA, IglC, and pdpA are also required for escape of *F. tularensis* into the host cells cytosol but it has not been determined if they are secreted substrates or component of the type VI-like secretion apparatus.

Mutations in MglA and FevR negatively affect the ability of *F. tularensis* to escape from the phagosome into the cytosol (Santic et al., 2005b; Bonquist et al., 2008; Buchan et al., 2009). In contrast, MigR mutant behaves similar to the IglD mutant, which escapes but is unable to replicate within the cytosol indicating that MigR regulate genes that are important for replication within the cytosol (Santic et al., 2007; Buchan et al., 2009). Other genes that play critical roles in the escape of bacteria into the host cell cytosol include genes involved in DNA modification, transcription and translation, type II secretion, metabolic genes as well as genes involved in the transport of nutrients (Schulert et al., 2009; Asare and Abu Kwaik, 2010).

Although hemolytic activity has been observed in *F. tularensis* subspecies *novicida* and *F. philomiragia* (Lai et al., 2003), no hemolysin homolog has been identified in all the sequenced *Francisella* genome to date including those of *novicida* and *philomiragia*. There are between four and eight acid phosphatases in the *Francisella* genome depending on the subspecies. There are eight acid Phosphatases in the subspecies *novicida* genome, four (AcpA, AcpB, AcpC, and HAP) of which are also found in the virulent subspecies *tularensis* genome (Mohapatra et al., 2008). AcpA has

been shown to possess lipase activity (Mohapatra et al., 2007a), but all three Acp molecules are predicted to possess phosphoric ester hydrolase activity. Independent studies have shown that mutations in AcpA, AcpC, and HAP result in delay or inhibition of escape into the cytosol and reduced replication within human macrophages (Mohapatra et al., 2007a; Asare and Abu Kwaik, 2010), and that combined deletion of AcpA, AcpB, AcpC, and HAP results in complete inhibition of phagosomal escape and replication of subspecies *novicida* in the cytosol (Mohapatra et al., 2008). However, there is contradictory data on the role of these acid phosphatases in escape and intracellular replication. For example, Baron et al. (1999) have shown that AcpA in subspecies *novicida* is not important for replication within mouse macrophages. The difference between the role of AcpA in various subspecies may be due to the difference in the macrophages used, since trafficking of *F. tularensis* has been shown to be slightly different in mouse and human macrophages (Clemens et al., 2004; Checroun et al., 2006). Similarly, Child et al. (2010) have shown that combined deletion of AcpA-C does not affect the phagosomal escape or replication of the virulent subspecies *tularensis* within human macrophages. This indicates that there may be subtle differences in the mechanisms used by the different subspecies to escape into the host cell cytosol. There are numerous genes identified to be important for escape of *F. tularensis* that are designated as hypothetical proteins or proteins with unknown functions (Asare and Abu Kwaik, 2010). Some of these may be potential substrates for the type VI-like secretion system and identifying and characterizing them will help us to understand how *F. tularensis* modulates biogenesis of its phagosome and escape into the cytosol.

MODULATION OF INFLAMMATORY RESPONSE TO INFECTION BY *F. TULARENSIS*

The transcription factor NF-κB is involved in the regulation of inflammation by activating the induction of different proinflammatory cytokines (Lawrence, 2009). NF-κB represents a family of homo and heterodimer transcription factors, and the p65/p50 heterodimer is the most predominant active complex in mammalian cells (Burstein and Duckett, 2003). In resting cells, NF-κB proteins are predominantly sequestered in the cytoplasm by the NF-κB inhibitory proteins (IκBs; Karin and Ben-Neriah, 2000). The IκB kinase mediates phosphorylation of IκBs, followed by ubiquitination and proteasomal degradation, which is crucial to the activation and nuclear translocation of NF-κB (Karin and Ben-Neriah, 2000).

Early during infection when *F. tularensis* is localized within the phagosome, it activates the inflammatory response in macrophages by inducing the secretion of TNF-α in TLR-2 dependent manner (**Figure 2**; Telepnev et al., 2005; Katz et al., 2006). Induction of TNF-α secretion is mediated by the PI3K/Akt pathway, which also leads to activation of NF-κB (Telepnev et al., 2003; Katz et al., 2006; Parsa et al., 2006; Rajaram et al., 2006). Activation of NF-κB results in the induction and secretion of proinflammatory cytokines that restrict the escape of *F. tularensis* from the phagosome into the cytosol and promotes fusion of the FCP with the lysosome (**Figure 2**; Rajaram et al., 2009). Concomitant with escape into the cytosol, *F. tularensis* down-regulates NF-κB activation and TNF-α, IL-6, IL-8, and IL-12 secretion within 5 h post-infection, since the *IglC* mutant which is unable to escape into the cytosol, does not down-regulate TNF-α, IL-6, IL-8, and IL-12 secretion (**Figure 2**; Telepnev et al., 2003, 2005).

The activation of PI3K/Akt pathway is negatively regulated by the Src homology 2 (SH2) domain-containing inositol-5′-phosphatase (SHIP) protein, since deficiency in SHIP expression results in enhanced Akt activation and NF-κB-driven transcription of proinflammatory cytokines, which promote fusion of the FCP with the Lysosome (**Figure 2**; Parsa et al., 2006; Rajaram et al., 2009). Conversely, over expression of SHIP leads to a decrease in NF-κB activation (Parsa et al., 2006). It is unknown how the delicate balance of Akt and SHIP activation is tilted toward SHIP promoted escape of *F. tularensis* into the cytosol. It will be interesting to determine how *F. tularensis* activates SHIP and the relations between SHIP activation and the disruption of the phagosome membrane that allow *F. tularensis* to escape into the cytosol (**Figure 2**). Once inside the cytosol *F. tularensis* induces Sp-1/Sp-3 dependent Fas expression that results in activation of caspase-3 and host cell death (Rajaram et al., 2009).

Cytosolic localization of *F. tularensis* in mouse macrophages results in type I interferon (IFN-I) and AIM2 dependent activation of the inflammasome (**Figure 2**; Mariathasan et al., 2005; Gavrilin et al., 2006; Henry et al., 2007; Fernandes-Alnemri et al., 2010; Jones et al., 2010). Cytosolic bacteria induce IRF-3 dependent activation of IFN-I, which in turn increases the expression of AIM2 (**Figure 2**; Jones et al., 2010). AIM2 recognize *F. tularensis* (Fernandes-Alnemri et al., 2010), likely through binding to dsDNA from the bacteria since *F. tularensis* DNA co-localizes with AIM2 (Jones et al., 2010). Upon binding to *F. tularensis* DNA, AIM2 forms a complex with the adapter protein ASC and caspase-1 known as the inflammasome (Mariathasan et al., 2005; Fernandes-Alnemri et al., 2010; Jones et al., 2010). Inflammasome activation by *F. tularensis* results in the formation of pyropoptosome, which is critical for innate host defense and leads to pyropoptotic death of infected cells and the concomitant release of the proinflammatory cytokines IL-1β and IL-18 (**Figure 2**; Mariathasan et al., 2005; Gavrilin et al., 2006). The *F. tularensis* lipid/polysaccharide (MOP) transporter protein, MviN, which is homologous to the *E. coli* putative lipid II flippase, has recently been shown to suppress the induction of AIM2 (Ulland et al., 2010), since a mutation in the gene results in increase induction of AIM2 inflammasome-dependent IL-1β secretion and cytotoxicity in macrophages (Ulland et al., 2010). In addition to MviN, two other genes FTT_0584, with no characterized orthologs, and FTT_0748, which is homologous to the IclR family of transcriptional regulators, have been shown to suppress caspase-1 and ASC dependent secretion of IL-1β, since mutations in these genes resulted in hyper secretion of IL-1β (Weiss et al., 2007). Since AIM2 is not present in human macrophages, inflammasome mediators are likely to be different from the one described for mouse macrophages.

ACTIVATION AND CONTROL OF HOST CELL APOPTOSIS

Between 6 and 12 h post-infection, *F. tularensis* induce caspase-3 activation within the host cells, which culminate in the induction of apoptosis (Lai and Sjostedt, 2003; Santic et al., 2010b). *F. tularensis* LVS induces apoptosis in the J774A.1 murine macrophage cell

line through a pathway partly resembling the intrinsic apoptotic pathway (Lai and Sjostedt, 2003). The induction of apoptosis involves the release of mitochondrial cytochrome C into the cytosol with concomitant activation of caspase-9 and caspase-3 but not caspase-1, caspase-8, Bcl-2, or Bid (Lai and Sjostedt, 2003). In contrast, another study has shown that *F. tularensis* induces Sp-1/Sp-3 activation of Fas in RAW 264.7 murine macrophage cells line, which results in activation of caspase-3, suggesting that *F. tularensis* induce apoptosis through the extrinsic pathway (**Figures 1 and 2**; Rajaram et al., 2009). Interestingly, infection of murine macrophages by *F. tularensis* has been shown to induce apoptotic cell death through down-regulation of activation of p38 MAPK compared to uninfected cells, but the mechanism of induction is yet to be defined (Hrstka et al., 2005). Although caspase-3 activation occurs early during infection in non-activated macrophages, it is not until about 18 h post-infection before there is induction of apoptosis, which is likely due to triggering anti-apoptotic processes (**Figures 1 and 2**; Lai and Sjostedt, 2003; Al-Khodor and Abu Kwaik, 2010; Santic et al., 2010b).

HOST FACTORS REQUIRED INTRACELLULAR GROWTH OF *F. TULARENSIS*

NF-κB plays a crucial role in regulation of apoptosis by triggering expression of various anti-apoptotic genes (Burstein and Duckett, 2003). We have shown that in order to maintain viability of the infected cell and allow *F. tularensis* to survive, there is simultaneous activation of caspases and NF-κB creating a delicate balance between them to maintain cell viability that is necessary for proliferation of the bacterium. Activation of NF-κB involves IκB kinase-mediated phosphorylation of IκBs, followed by ubiquitination and proteasomal degradation (Karin and Ben-Neriah, 2000). Interestingly, in activated macrophages, *F. tularensis* elicit ubiquitin-dependent MHC class II down-regulation and degradation, thus compromising antigen presentation by macrophages to CD4 T cells (Wilson et al., 2009). It is not surprising that two ubiquitin proteins, the ubiquitin hydrolase USP22, and the ubiquitin ligase CDC27 has been shown to be important for replication of *F. tularensis* in human macrophages (Akimana et al., 2010).

It has been shown that *F. tularensis* triggers activation of Ras through the recruitment of PKCα and PKCβ-I to the SOS2/GrB2 complex (**Figure 2**; Al-Khodor and Abu Kwaik, 2010). Silencing of SOS2, GrB2, PKCα, and PKCβ-1 is associated with rapid early activation of caspase-3 but does not affect phosphorylation of Akt or Erk (Al-Khodor and Abu Kwaik, 2010). This indicates that *F. tularensis* utilizes two independent mechanisms to modulate caspase-3 activity in order to survive inside host cells till the terminal stages of infection when induction of apoptosis leads to cell lysis and release of bacteria to the extracellular milieu. The bacterial factor necessary for the activation of NF-κB and Ras are yet to be identified.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Upon infection with *F. tularensis*, the host cells employ a myriad of arsenals to try to limit proliferation of the bacteria. The host cells activate signaling pathways to try to restrict escape of *F. tularensis* into the cytosol. Once the bacteria escape into the cytosol, a new arsenal is put into motion by the host cells through activation of caspase-1 and caspase-3, geared toward pyropoptosis and apoptosis of the infected cells. Concomitantly, there is activation of NF-κB geared toward triggering pro-survival signals and the induction of proinflammatory cytokines. Intuitively, *F. tularensis* has devised different strategies to counteract the innate host defense mechanisms. These include inhibition of components of the host defense mechanism and hijacking the cells own defense system and other signaling pathways through bacterial effectors that are likely exported through a type VI-like secretion system. For example, *F. tularensis* co-opts the host cell NF-κB transcription factor, which is used to activate proinflammatory cytokines, to induce the expression of anti-apoptotic genes to maintain cell viability. Similarly, *F. tularensis* co-opts the host cell Ras signaling pathway to inhibit caspase-3-induced apoptosis. Finally, *F. tularensis* utilizes the host cell ubiquitin-dependent proteasome degradation system to degrade MHC class II molecules on activated macrophages to inhibit antigen presentation to effector T cells.

Many virulence factors have been identified that are required for bacterial escape and replication within the cytosol. Although some of these are involved in known pathways, majority of these have no known functions. The roles of some of these factors in the virulence mechanisms exhibited by *F. tularensis* are beginning to be defined but the functions are largely unknown. Cytosolic *F. tularensis* activates PKC leading to activation of Ras and inhibition of apoptosis. MigR regulates genes that are important of phagosome biogenesis. Identifying and characterizing MigR-regulated genes will lead to an understanding of how *F. tularensis* arrest phagosome maturation. Delineating how MviN, FTT0584, and FTT0748 inhibit caspase-1 activity will shed light on how *F. tularensis* modulate pyropoptosis and proinflammatory cytokine induction. Since NF-κB is required for both cytokine induction and inhibition of apoptosis, its activation must be tightly controlled. It will be interesting to identify the bacterial factors important for activation of Ras and diversion of NF-κB to the expression of anti-apoptotic effectors. It is not known how *F. tularensis* tilts the balance of power between Akt and SHIP toward SHIP activation and bacterial escape and the mechanism by which this is achieved. Unlike *L. monocytogenes*, no hemolysin-like molecule have been identified in *F. tularensis* and there is contradictory data on role of AcpA in escape, which may be partly due to the studies being done using different species of *Francisella* and different sources of macrophages. It will be interesting to know if SHIP does not only inhibit cytokine activation but also activate a host cell factor that leads to disruption of the phagosome membrane and escape of the bacteria. It will be interesting to determine how *F. tularensis* modulate the ubiquitin ligase in activated macrophages leading to degradation of MHC II molecules and evasion of adaptive immunity. There is little doubt that *F. tularensis* employs various strategies to modulate cellular processes that have evolved to degrade invading microbes in addition to evasion of various innate and adaptive immune processes to inflict disease in the mammalian host. It is just as interesting to uncover the molecular and cellular bases of the interaction of *F. tularensis* with the arthropod vector and its role in pathogenic evolution and infection of the mammalian host. It is likely that the pathogen exploits conserved eukaryotic processes to infect evolutionarily distant hosts as well as processes unique to the infection of mammals.

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